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An Alternative Tissue Culture Media for Potato (*Solanum tuberosum* L.) Micro Propagation

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Abstract

The cumbersome nature of tissue culture technique contributes to the low adoption of the technique. This work aims at developing an accessible alternative tissue culture medium for potato micro propagation. The inorganic components of the conventional MS medium were replaced with a common source of nitrogen, phosphorus and potassium (NPK) to regenerate *in vitro* plantlets of six improved Cameroonian potato varieties selected from meristem-derived plantlets. These varieties included: Cipira, Mafo, Bambui wonder, Irad 2005, Jacob 2005 and Tubira. Five (5) nodes each per replicate per variety were cultured on both media and allowed to grow at a temperature of 21 ºC and 16hrs/day photoperiod for 28 days.

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Number of nodes sprouting, number of nodes rooting, plant height, and number of nodes per plantlet were recorded weekly in 4 weeks. Results show that both media regenerated vigorous plantlets with shoots and roots. Using the Statistical Package for Social Science (SPSS), analysis of variance revealed slight significant differences ($p \leq 0.05$) between varieties and media in terms of sprouting and rooting Height of plantlets on NPK medium ranged from 29.85 mm (Cipira) to 45.25 mm (Irad 2005) meanwhile, on MS medium it ranged from 47.93 mm (Bambui Wonder) to 65.70 mm (Jacob 2005). Average number of nodes ranged from 2.59 (Tubira) to 3.63 (Mafo) on fertilizer medium while on MS medium it ranged from 2.70 (Irad 2005) to 4.20 (No significant difference at $p \leq 0.05$). in conclusion; the developing countries can replace the inorganic components of MS medium with inorganic fertilizer (NPK) for *in vitro* multiplication of potato.

Keywords: *Solanum tuberosum*; nodal culture; MS medium; inorganic fertilizer medium.

1. Introduction

One of the Millennium Development Goals (MDG) of the Food and Agriculture Organisation (FAO) was to reduce chronic hunger [1]. Today, the Sustainable Development Goals of the United Nations hold that agriculture will play a crucial role in addressing the planet's future needs in food production [2]. The maximum utilization of Africa's crop resources will therefore contribute a great deal in achieving this goal. Potato is the first largest cultivated root and tuber crop and fourth amongst the world's most important food crop after wheat, maize and rice [3]. It is rich in proteins, essential vitamins, minerals, trace elements, very low fat content and even medicinal properties [4]. According to FAO statistics [2], the demand for potato in Africa outweighs that of Europe. Despite this fact, the production figures of potatoes in Africa are still generally very low (16,706,573 tons/year) compared to that of Europe (130,343,664 tons/year). One of the major production constraints in African countries (Cameroon) has been identified as the unavailability of disease-free planting material [5]. This could be attributed to the low adoption of modern techniques of producing disease-free planting material. The basis of agricultural production and nutrition of mankind is seed quality. Seed is, in agricultural terms, any materials that are used to plant crops [6]. In seed programmes it is essential to initiate crop production with disease-free seeds since diseases affect the seed quality and yield. Tissue culture techniques such as meristem culture is therefore at the entrance of most potato seed programmes to obtain disease-free seeds [7,8,9]. After obtaining disease-free *in vitro* plantlets, the use of nodal culture as a multiplication technique is inevitable [10]. Despite the role tissue culture plays in disease elimination, rapid multiplication and germplasm conservation, the cost of the medium (inorganic nutrient components) is still a hold up in potato production in the developing world [11]. The use of common sources (inorganic fertilizers) of both the macro and micro nutrients is possible for tissue culture and could reduce cost to about 92.2 %. Plant varieties differ in their response to the inorganic fertilizer used in the place of the Murashige and Skoog (MS) iron source, macro and micro nutrients [12]. The success of tissue culture depends so much on the culture medium as no single medium will support the growth of all cells and changes in the medium are often necessary for different types of growth responses as observed by [13]. There are several formulations of the basic tissue culture nutrient medium. The most widely used is the Murashige and Skoog (MS) formulation [14]. Among all these formulations, only the concentrations and quantities of the inorganic salt components are being modified, sometimes the hormonal concentration. These components are often not readily available and not easy to import for developing countries. Affording for them at times entails placing and order and waiting for long periods time

to receive. And these are some of the hold ups in the adoption of tissue culture techniques in developing countries.

1.1. Significance of the study

To improve on the adoption of tissue culture technique through the development of an alternative tissue culture medium for rapid multiplication of seed potato using a common source of Nitrogen, Phosphorus and Potassium (NPK).

Hypothesis

Null: Common sources of NPK will have no significant effect on the growth performance of potato explants cultured *in vitro*.

Alternative: Common sources of NPK will have a significant effect on the growth performance of potato explants cultured *in vitro*.

2. Materials and method

The plant material used in this study included meristem-derived plantlets of 6 improved potato varieties (Cipira, Mafo, Jacob 2005, Bambui wonder, Irad 2005 and Tubira) in the Biotechnology laboratory of the Institute of Agricultural Research for Development (IRAD) in Cameroon. The NPK media was prepared by replacing the conventional MS medium with an inorganic fertilizer with composition N (20), P (10), K (10). Vigorous plantlets were selected and cut inter node in a laminar air flow chamber (figure 1). Five nodes per replicate were cultured in four replications on both NPK and MS media in test tubes. The conventional MS medium and fertilizer medium were both supplemented with 2.5% sucrose, 0.1g l^{-1} myo-inositol, 1 ml l^{-1} of 1mg ml^{-1} solution of GA_3, 6g l^{-1} of agar and pH adjusted to 5.6. The tubes were sealed, labeled accordingly and incubated in a growth room at a temperature of 21 °C and 16 hrs/day photoperiod. Data was recorded weekly for a period of four weeks on the number of nodes sprouting, rooting, average plant height and average number of nodes. Analysis of variance was done using Statistical Package for Social Science (SPSS) version 17.0 to ascertain the differences between the media and varieties. Means were separated using the Turkey's HSD test at 5% significance level.

Figure 1: Nodes of *in vitro* plantlets cut prior to culture on both media

3. Results

All the six potato varieties regenerated plantlets with roots and shoots on both media after a growth period of 4 weeks. Five (Cipira-C, Jacob 2005-J, Irad 2005-I, Bambui wonder-BW, and Mafo-M) of the six varieties showed no significant difference at P≤0.05 between the two media in terms of number of nodes sprouting (Table 1). Mean number of nodes sprouted (NNS) out of the 5 cultured per replicate ranged from 4.88 to 5.00 on both media. No significant difference was also noticed amongst varieties at $P \leq 0.05$. The variety Tubira-T showed a significant difference between Fertilizer medium and MS medium.

Table 1: Mean NNS of six potato varieties on MS and Fertilizer medium.

Varieties Medium	BW	T	J	C	I	M
MS	5.00^{ax}	4.75^{bx}	5.00^{ax}	5.00^{ax}	5.00^{ax}	5.00^{ax}
Fertilizer (F)	4.75^{ax}	5.00^{ax}	4.88^{ax}	5.00^{ax}	4.88^{ax}	5.00^{ax}

Means having the same letters are not significantly different using the Tukey's HSD at 5% level; **ab** represents comparisons within rows while **x** represents comparisons within columns.

In the case of mean number of nodes rooting (NNR), there were significant differences noticed between media and between varieties (Table 2). All the potato varieties were able to form roots on both media at the end of 4 weeks growth period.

Table 2: Mean NNR of six potato varieties on MS and Fertilizer medium.

Varieties Medium	BW	T	J	C	I	M
MS	4.44^{abx}	4.75^{ax}	4.69^{ax}	4.00^{ax}	4.94^{ax}	5.00^{ax}
Fertilizer (F)	4.38^{cx}	5.00^{ax}	4.06^{cx}	4.44^{abcx}	4.81^{abx}	5.00^{ax}

Means having the same letters are not significantly different using the Tukey's HSD at 5% level; **abc** represents comparisons within rows while **x** represents comparisons within columns.

The average number of nodes (ANN) per replicate ranged from 2.70 (J) to 3.63 (M) on Ferilizer (F) medium and with a similar trend observed on the MS medium ranging from 2.70 (I) to 4.02 (M) on the MS medium. There was no significant difference noticed between varieties and media at $P \leq 0.05$ (Table 3).

Table 3: ANN of six potato varieties on MS and Fertilizer medium.

Varieties Medium	BW	T	J	C	I	M
MS	3.38^{ax}	3.93^{ax}	3.83^{ax}	3.49^{ax}	2.70^{ax}	4.02^{ax}
Fertilizer (F)	2.99^{ax}	2.59^{ax}	2.70^{ax}	3.40^{ax}	2.71^{ax}	3.63^{ax}

Means having the same letters are not significantly different using the Tukey's HSD at 5% level; **a** represents comparisons within rows while **x** represents comparisons within columns. The analysis of variance (ANOVA) revealed no significant difference between media and varieties in terms of average plant height (Fig. 2). Plant height ranged from 47.93 (BW) to 65.70 (J) (Fig 3).

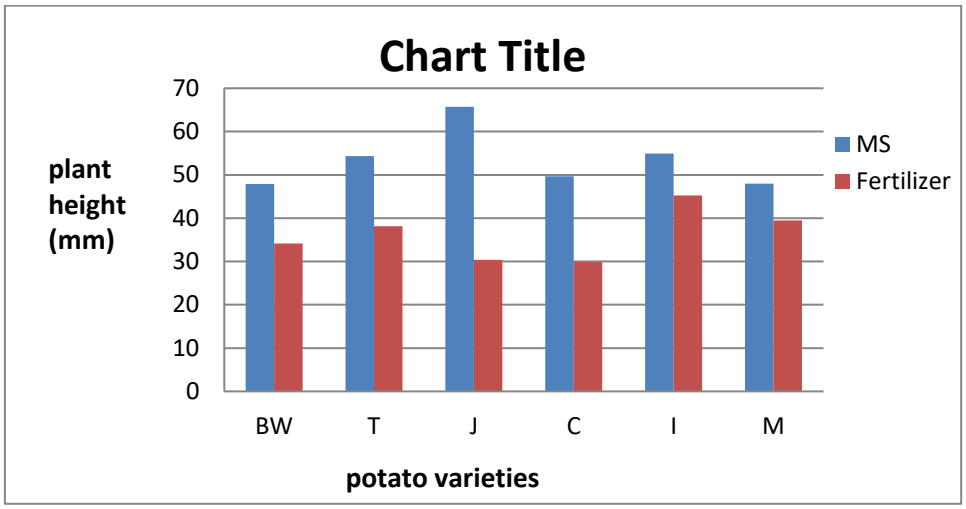

Figure 2: Average plant height of six potato varieties on MS and Fertilizer medium 4 weeks after culture.

a **b**

Figure 3: Plantlets of potato variety (Tubira) obtained 4 weeks after nodal culture on MS medium (a) and plantlets of variety Tubira obtained 4 weeks after nodal culture on Fertilizer medium (b).

4. Discussion

The bud found between the leaf and the stem of a potato plant when placed on an appropriate medium is capable of breaking dormancy and growing into an individual plant [15]. The nodes of all varieties broke dormancy on both fertilizer medium and MS medium implying that fertilizer medium could be appropriate for nodal culture of potato *in vitro*. The slight significant difference noticed between the MS and Fertilizer media in this study could be attributed to the fact that the nutritional and hormonal condition of the medium greatly affects plant regeneration in tissue culture [16]. The high percentage of sprouting on Fertilizer medium could be due to the fact that fertilizer medium contains the macro elements (N, P and K), and hormones necessary for plantlet regeneration just as in the conventional MS Medium [16]. Therefore the most important requirements for *in vitro* potato regeneration are N, P and K. This could also mean that plants' requirements *in vitro* could be kept at this minimum level. The differences in the means of sprouted nodes among varieties could be due to genetic variation between the varieties. The challenge of differential response to tissue culture medium by various plant varieties remain not only for potato but also for other crops [17]. All varieties in this study could form roots after four weeks implying that they could be established *ex vitro* during acclimatisation since roots are very important in the uptake of soil nutrients during acclimatisation [18]. In this study, the fertilizer medium was able to stimulate root formation just as much as the MS medium. Plantlets with roots are therefore expected to be able to absorb all required nutrients when exposed to *in vivo* conditions. In terms of plant height, no significant difference was recorded.

This is in harmony with results obtained by [12] during his first week of culture and a change in pattern in the subsequent weeks. Meanwhile, in this study, No significant difference ($p=0.05$) was recorded between the two media in the subsequent weeks probably due to variation in response by different crops. This is in contrast with [12] where he observed significant differences between MS medium and low cost fertilizer medium and [17] who also reported differential response in node production of 27 sweet potato varieties during in *vitro* propagation. [19] Also reported some differences in the regeneration efficiency of cassava varieties IDEA87 and CM6740-7 on a low cost tissue culture medium developed in Colombia using Hydro Agri's fertilizer. Obtaining plantlets with adequate plant height and number of nodes on the fertilizer medium allows for subsequent subculture and thus, rapid multiplication just as on MS medium.

5. Conclusion

Plantlets of all the six improved potato varieties were successfully regenerated from nodal cuttings on the fertilizer medium in a similar way as on the MS medium. It is therefore possible to replace the inorganic salt components of MS medium with a common source of N, P and K in the ratio 20:10:10 respectively.

6. Recommendations

Developing countries could employ the use of any available source of Nitrogen, Phosphorus and Potassium as an alternative source of the inorganic salts of Murashige and Skoog basal medium for the rapid propagation of potato and other horticulture crops.

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